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Field Crops Research 99 (2006) 67–74
Field Crops Research
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Large applications of fertilizer N at planting affects seed protein and oil concentration and yield in the Early Soybean Production System☆

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Received 7 October 2005; received in revised form 23 March 2006; accepted 25 March 2006

Abstract

An inverse relationship between soybean [*Glycine max* (L.) Merr.] seed protein and oil concentration is well documented in the literature. A negative correlation between protein and yield is also often reported. The objective of this study was to determine the effect of high rates of N applied at planting on seed protein and oil. Nitrogen was surface-applied at soybean emergence at rates of 290 kg ha^{-1} in 2002, 310 kg ha^{-1} in 2003, and 360 kg ha^{-1} in 2004. Eight cultivars ranging from Maturity Group II–IV were evaluated under the Early Soybean Production System (ESPS). However, not all cultivars were evaluated in all 3 years. Glyphosate herbicide was used in all 3 years and a non-glyphosate herbicide treatment was applied in 2002. Cultivars grown in 2003 were also evaluated under an application of 21.3 kg ha^{-1} of Mn. All cultivar, herbicide, and Mn treatments were evaluated in irrigated and non-irrigated environments with fertilizer N (PlusN treatment) or without fertilizer N (ZeroN treatment). When analyzed over all management practices (years, cultivars, herbicide, and Mn treatments), the PlusN treatment resulted in a significant decrease in protein concentration (2.7 and 1.9%), an increase in oil concentration (2.2 and 2.7%), and a decrease in the protein/oil ratio (4.7 and 4.6%) for the irrigated and non-irrigated environments, respectively. However, the overall protein and oil yield increased with the application of fertilizer N at planting (protein: 5.0% irrigated, 12.7% non-irrigated and oil: 9.9% irrigated and 18.9% non-irrigated). These increases were due to the increase in seed yield with the application of large amounts of fertilizer at planting. Additionally, a significant correlation ($r = 0.45$, $P = 0.0001$) was found between seed protein concentration and seed yield. No significant correlation was found between seed oil concentration and seed yield. The data demonstrate the inverse relationship between protein and oil and indicate that large amounts of N applied at planting do not change this relationship.
Published by Elsevier B.V.

Keywords: Soybean; ESPS; Nitrogen; Fertilizer; Protein; Oil

1. Introduction

Soybean [*Glycine max* (L.) Merr.] can be used in a wide range of products from hand lotion to diesel fuel (USB, 2004), however, its primary use is as a high protein meal and secondarily as a vegetable oil source. Yaklich et al. (2002) analyzed 51 years of data from the Uniform Soybean Tests and reported that the mean seed protein and oil concentration for the Southern Region Test was 411 g kg^{-1} of protein and 209 g kg^{-1} of oil. However both protein and oil concentration can vary greatly with genotype and environment. In order for soybean to produce a high protein meal, a large amount of N is required. A 4000 kg ha^{-1} soybean crop has about 260 kg ha^{-1} of N in the harvested grain (Olson, 1978) as compared to 129 kg ha^{-1} in the harvested grain of a 9500 kg ha^{-1} corn crop (Barber and Olson, 1968; Olson, 1978). This difference in seed N concentration is reflected in seed protein concentration (≈410 g kg^{-1} soybean versus ≈100 g kg^{-1} corn).

The N source of soybean is a combination of symbiotic N_2 fixation and mineral N assimilation. Estimates vary, but N from N_2 fixation accounts for between 50 and 75% of the

Abbreviations: ESPS, Early Soybean Production System; DAP, days after planting; DTM, days to maturity; MG, maturity group

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0378-4290/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.fcr.2006.03.006

soybean demand for N (Hardarson et al., 1984; Bergersen et al., 1985; Matheny and Hunt, 1983; Unkovitch and Pate, 2000). Large amounts ($\geq$100 kg ha^{-1}) of N fertilizer applied to soybean have been variously reported to have no effect on yield (Welch et al., 1973; Freeborn et al., 2001; Gan et al., 2003; Gutiérrez-Boem et al., 2004), mixed effects (Lyons and Earley, 1952), or to increase yield (Weber, 1966; Sorensen and Penas, 1978; Purcell and King, 1996; Purcell et al., 2004; Ray et al., 2006). In general, reports of no effect on yield are those with post-flowering or late season applications of fertilizer N. Indeed, many of the reports of increased yield with fertilizer N also indicate an increased seed number per m^2 (Weber, 1966; Purcell and King, 1996; Ray et al., 2006) upon which late season N applications would have no effect. In addition to stage of development at which the N is applied, other factors such as temperature, soil water content, soil type, and organic matter content may affect the response to fertilizer N on soybean.

The effects of large amounts of fertilizer N on protein and oil concentrations of soybean seed are not as well documented. Weber (1966) reported small increases in seed protein concentration and decreases in oil concentration with 56 and 168 kg ha^{-1} of N applied at planting. Purcell et al. (2004) reported a general trend of decreasing protein concentration and increasing oil concentration with 112 kg ha^{-1} of N applied at V6, full bloom (R2), or V6 and R2 (stages according to Fehr et al., 1971). Gutiérrez-Boem et al. (2004) found that late season (beginning pod, R3 and beginning seed fill, R5) applications of fertilizer N (50 or 100 kg N ha^{-1}) had no effect on protein concentration. On the other hand, maintenance of N_2 fixation through full pod (R6) has been associated with high seed–protein concentration (Leffel et al., 1992; Fabre and Planchon, 2000). Although normally not recommended as an economical practice, application of fertilizer N on soybean can be used to provide N at higher levels and/or for longer periods than can be achieved through symbiotic N-fixation alone. In this report we describe the effect of large amounts of fertilizer N (290–360 kg ha^{-1}) applied at planting on soybean protein and oil concentration for irrigated and non-irrigated Early Soybean Production System (ESPS) planting at Stoneville, MS in 2002, 2003, and 2004.

2. Materials and methods

In order to describe the effects of large fertilizer N applications on soybean across a broad range of conditions, a series of experimental factors were included in this study. An overview of the experiment can be found in Table 1 (from Ray et al., 2006 and is reproduced here to orient the reader). Ray et al. (2006) provides a more detailed discussion of the experimental design and conduct, however the essential materials and methods are summarized here. Field experiments with four replications were conducted over a 3-year period (2002–2004). In each year, either no fertilizer N (ZeroN treatment) or large amounts of fertilizer N (PlusN treatment) were applied to soybean grown in irrigated and non-irrigated environments. As shown in Table 1, weed populations were managed with either non-glyphosate or glyphosate treatments in 2002, whereas, in 2003 and 2004 only the glyphosate treatment was used. A granular, surface applied, Mn fertilizer treatment (21.3 kg ha^{-1}) was applied in 2003 but not in 2002 and 2004 (Table 1). In each year four cultivars were grown, however, not every cultivar was grown every year (Table 1). To simplify discussion, years, herbicide treatments, Mn treatments, and cultivars are collectively referred to as ''management practices''. Data analysis was

Table 1
Description of the factors analyzed over the 3 years of the study

MG[a]	Management practices (random effects)[b]				Fixed effects[c]	
	Cultivar	Year grown[d]	Herbicide[e]	Added Mn[f]	Irrigation[g]	Added nitrogen[h]
II	A 2703	2002	G,C	N	N,I	P,Z
III	A 3702	2003, 2004	G	Y,N	N,I	P,Z
IV	A 4702	2002–2004	G,C	Y,N	N,I	P,Z
IV	AP 4882	2002	G,C	N	N,I	P,Z
III	DK 3964	2003, 2004	G	Y,N	N,I	P,Z
IV	HBK 4820	2003	G	Y,N	N,I	P,Z
IV	HBK 4920	2004	G	N	N,I	P,Z
II	Jack	2002	G,C	N	N	P,Z

Although some factors varied from year to year, all were replicated four times and were evaluated with and without fertilizer N (ZeroN and PlusN treatments).
[a] Maturity Group classification.
[b] In the text, cultivar, year, herbicide and Mn treatments are collectively referred to as ''Management Practices'' which were treated statistically as random effects.
[c] Irrigation environment and nitrogen treatment were treated statistically as fixed effects.
[d] Year(s) of the study when the cultivar was evaluated.
[e] Herbicide treatments, G = glyphosate all 3 years, C = conventional in 2002.
[f] Whether or not the cultivar was grown with the additional treatment of 23.1 kg ha^{-1} of Mn in 2003, Y = Yes and N = No.
[g] Irrigation environment under which the cultivar was grown, N = non-irrigated and I = irrigated.
[h] Added N, P = PlusN treatment (290 kg ha^{-1} in 2002, 310 kg ha^{-1} in 2003 and 360 kg ha^{-1} in 2004), Z = ZeroN treatment (no fertilizer N).

conducted across all management practices and irrigation environments to determine the effect of adding large amounts of fertilizer N to soybean.

Soybean was grown on Sharkey clay soil (very-fine, smectitic, thermic, Chromic, Epiaquert) at the Delta Research and Extension Center at Stoneville, MS (latitude 33°26′N). Sharkey is the dominant soil series in the lower Mississippi River Valley alluvial flood plain, and residual mineral N is low on these clayey soils in the midsouthern USA. Soil analyses indicated that the pH ranged from 6.5 to 7.7, and that no supplemental P and K were necessary (Varco, 1999; Heatherly and Elmore, 2004). The non-irrigated environment was at the same location each year. The irrigated environment was in different locations each year but always within 200 m of the non-irrigated site. All sites had a common, uniform soil type and had been in continuous soybean for 20 years with each environment having a common irrigation history (either irrigated or non-irrigated) for the past 10 years.

In normal soybean production on these soils, N-fertilizer is rarely applied and is not recommended. The N source for the crop is residual N and symbiotic N-fixation. Soil analysis at the time of planting in 2003 indicated nitrate concentrations in these soils of 9.1 kg ha^{-1} (n = 128, S.D. = 1.4) as determined in a commercial soils laboratory (Pettiet Agricultural Services Inc., Leland, MS) and reflects the low levels of residual N in these soils. In these experiments, it was expected that the high rates of N-fertilizer (>290 kg ha^{-1}) applied would reduce N-fixation (Harper and Gibson, 1984; Gibson and Harper, 1985). Although, N-fixation was not measured, we previously reported that ureide concentrations in aboveground biomass were significantly reduced (57.2% irrigated and 53.5 % non-irrigated) by these rates of applied N (Ray et al., 2006). Ureides are the N-transport molecules from the nodules to the leaves and these significant reductions clearly indicate a major effect on nodulation and/or nodule activity of the applied N-fertilizer.

The experiment was laid out as a randomized complete block design with four replications and a split-plot factorial arrangement of the treatments, with cultivar as the main plot and N rate as the subplot. The result of the design was that each management practice was grown with or without fertilizer N (PlusN and ZeroN treatments) and with or without irrigation. In the non-irrigated environment, treatments were randomly assigned to plots in 2002 and remained in the same location for 2003 and 2004. In the irrigated environment, treatments were randomly assigned at the beginning of each year. The field was prepared by a shallow (<10 cm deep) pass with a disk harrow and spring-tooth cultivator in the preceding fall and existing weeds were killed with a preplant application of glyphosate (840 g a.i. ha^{-1} in 94 L of water ha^{-1}). Seeding occurred on 17 April 2002, 2 April 2003, and 25 March 2004 into the stale seedbed (Heatherly, 1999) at a rate of approximately 16 seed m^{-1} of row, corresponding to about 50 kg of seed ha^{-1}. Rows were 0.5 m apart and plots were eight rows wide and 22 m long. Cultivars ‘A 2703’ and ‘Jack’ (Maturity group II) and ‘A 4702’ and ‘AP 4882’ (MG IV) were sown in 2002, and ‘DK 3964’ and ‘A 3702’ (MG III) and A 4702 and ‘HBK 4920’ (MG IV) were grown in 2003 and 2004 (Table 1). Selection of cultivars was based on regional variety trial results, use patterns by producers, recency of release, and the duration of their growing season, with MG II and MG III cultivars chosen for their short growing season (MG II: 110 days to maturity [DTM]; MG III: 120 DTM), and MG IV (133 DTM in 2002, 138 DTM in 2003, and 145 DTM in 2004) to represent a normal growing season length in the ESPS. Mefenoxam [(R)-2-{2,6-(dimethylphenyl)-methoxyacetylamino}-propionic acid methyl ester] fungicide at 0.11 g a.i. kg^{-1} was used to treat seed prior to planting each year.

The two N treatments were selected to represent current management practice in the ESPS (ZeroN treatment) and enough fertilizer N to support a seed yield of at least 4700 kg ha^{-1} (PlusN treatment). In the PlusN treatments surface applications of granular ammonium nitrate (340 g N kg^{-1} material) amounted to N levels of 290 kg ha^{-1} (24 April 2002), 310 kg ha^{-1} (16 April 2003), and 360 kg ha^{-1} (22 April 2004). In each year, rainfall of >2 cm occurred no later than 10 d after N application. Additional weather data applicable for this study were previously summarized (Ray et al., 2006).

The weed populations were controlled with post-emergence applications of appropriate herbicides. In the irrigated environment, the initial irrigation was conducted just before or after beginning pod (3 June 2002, 4 June 2003, and 9 June 2004) and was followed by additional irrigations whenever soil water potential at the 30-cm depth decreased to about −50 kPa (tensiometer readings). Irrigation was conducted using furrow irrigation and continued through full seed (R6) of all cultivars.

All cultivars were harvested within 5 days of maturity using a field combine modified for small plots. The four center rows of each plot were harvested between 5 and 30 August in 2002, between 28 July and 21 August 2003, and between 3 and 23 August 2004. Seed yield and two 100-seed samples collected from each plot were adjusted to 130 g kg^{-1} moisture content. Protein and oil concentrations were determined on a subsample taken from the yield samples. Analyses were conducted using NIR at the New Crops Processing laboratory (NCAUR-USDA-ARS, Peoria, IL).

Data were analyzed using analysis of variance [SAS PROC MIXED, Version 9 (Littell et al., 1996)] to determine the overall effect of the fertilizer N application. Management practices (years, Mn application, herbicide application, and cultivars) were treated as random effects. All management practices were applied to plots grown in two separate irrigation environments. Irrigation was treated as a fixed effect. While irrigation environments cannot be directly compared, the interaction between irrigation environment and nitrogen application can be evaluated for the consistency of the nitrogen response. Replicate plots (four each) of the management practices were considered subsamples of paired samples with or without fertilizer N. This analysis was not designed to evaluate effects on individual cultivars or management practices but to provide an estimate of an overall effect of applying large amounts of fertilizer N across a diverse range of conditions (irrigation environment, weed management, Mn fertilization, and cultivars).

3. Results

Protein and oil concentrations (g kg^{-1}) were determined from a subsample of each seed yield sample. Protein concentration ranged from 357.1 to 434.2 g kg^{-1} in the non-irrigated environment and from 361.5 to 428.0 g kg^{-1} in the irrigated environment. Fig. 1 shows a one-to-one graph of the protein concentration of the PlusN treatment plotted against the ZeroN treatment over all years, management practices, irrigation environments and N treatments of the 3-year study. For the irrigated environment, all 34 data points fell below the 1:1 line indicating a consistent decrease in seed protein concentration with the addition of fertilizer N at planting. Similar results were found in the non-irrigated environment except that 5 of 18 data points fell above the 1:1 line. There were no management practice consistencies among these five data points except that four of the five points occurred in the 2002 experiment. Nonetheless, when considered over all management practices (years, cultivars, herbicides and Mn treatments) there was a significant ($P < 0.0085$) 1.9% decrease in protein concentration (397.0 g kg^{-1} ZeroN versus 389.3 g kg^{-1} PlusN) in the PlusN treatment compared to the ZeroN treatment in the non-irrigated environment. A similar significant ($P < 0.0005$) 2.7% decrease (408.0 versus 397.2 g kg^{-1}) was found in the irrigated environment. There was no significant difference ($P = 0.4316$) in the response of protein concentration to fertilizer N between irrigation environments (Table 2).

The response of seed oil concentration was opposite to that of seed protein concentration. In the irrigated environment, oil concentration ranged from 203.3 to 241.6 g kg^{-1} and in the non-irrigated environment from 205.2 to 237.2 g kg^{-1}. Fig. 2 shows a one-to-one graph of the oil concentration of the PlusN treatment plotted against the ZeroN treatment over all years, management practices, N treatments and irrigation environments. Almost all data points fell above the 1:1 line indicating that added N tended to increase the oil concentration. In the irrigated environment, 4 of 17 data points fell on or below the 1:1 line and in the non-irrigated environment 5 of 18 data points fell below the 1:1 line. In the irrigated environment there were no management practice consistencies among the four data points but all five of the data points in the non-irrigated environment were from the 2002 experiment. However, when considered over all management practices (years, cultivars, herbicides and Mn treatments) there was a significant 2.2% increase ($P = 0.0045$) in oil concentration in the irrigated environment in response to fertilizer N (222.0 g kg^{-1} ZeroN versus 226.8 g kg^{-1} PlusN). In the non-irrigated environment there was a similar significant 2.7% increase ($P < 0.0006$, 217.0 g kg^{-1} ZeroN versus 222.8 g kg^{-1} PlusN) with fertilizer N. As with protein, the effect of fertilizer N on oil concentration was not significantly different ($P = 0.6590$) between irrigation environments (Table 2).

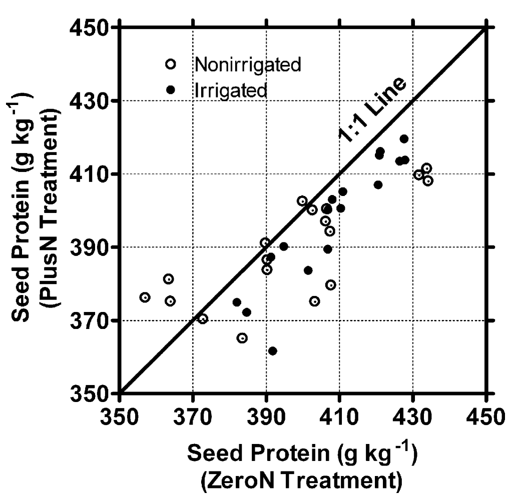

Fig. 1. One-to-one graph of protein concentration (g kg^{-1}) in the ZeroN treatment (x-axis) plotted against protein concentration in the PlusN treatment (y-axis) across all management practices (years, cultivars, herbicide, and Mn treatments) and irrigation environments (irrigated and non-irrigated). Each data point represents paired samples (i.e. the value of both a ZeroN and PlusN sample).

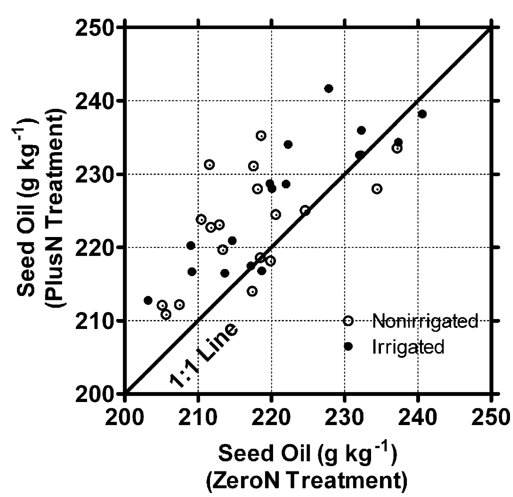

Fig. 2. One-to-one graph of oil concentration (g kg^{-1}) in the ZeroN treatment (x-axis) plotted against oil concentration in the PlusN treatment (y-axis) across all management practices (years, cultivars, herbicide, and Mn treatments) and irrigation environments (irrigated and non-irrigated). Each data point represents paired samples (i.e. the value of both a ZeroN and PlusN sample).

Table 2
Fixed effects and components of error of the analysis of variance (Proc Mixed, SAS)

Fixed effects	DF	Protein concentration		Oil concentration		Protein/oil ratio		Protein yield		Oil yield	
		F	Prob > *F*	*F*	Prob > *F*	*F*	Prob > *F*	*F*	Prob > *F*	*F*	Prob > *F*
Irrigation[a]	1	2.74	0.1072	2.46	0.1264	0.03	0.8686	32.93	<0.0001	37.44	<0.0001
N	1	22.08	<0.0001	23.14	<0.0001	29.58	<0.0001	81.46	<0.0001	252.58	<0.0001
Irrigation × N	1	0.63	0.4316	0.20	0.6590	0.01	0.9111	5.00	0.0322	3.22	0.0819

Components of error (random effects)[b]	Error	%	Error	%	Error	%	Error	%	Error	%
σ^2 (MP[c])	2.50	67.69	0.60	57.17	0.016	69.07	80003.0	89.17	21789.0	88.98
σ^2 (N × MP)	0.51	13.74	0.13	12.70	0.003	13.98	495.7	0.55	85.7	0.35
σ^2 (e)	0.68	18.57	0.31	30.13	0.004	16.95	9216.4	10.27	2613.3	10.67
Total	3.69	100.00	1.04	100.00	0.024	100.00	89715.1	100.00	24488.0	100.00

Years, Mn application, herbicide application, and cultivars were collectively analyzed as management practices (MP) and were treated as random effects. All management practices were grown in separate irrigation environments (irrigated and non-irrigated), which was treated as a fixed effect. N treatments consisted of either large amounts of fertilizer N applied at planting (PlusN) or no applied fertilizer N (ZeroN).

[a] ANOVA results for irrigation environment are shown for completeness. Direct comparison between irrigation environments was not statistically valid because of the structure of the experimental design.

[b] Proc Mixed of SAS was used and therefore components of variance are given instead of mean squares for random effects.

[c] MP = Management practices which consists of years, herbicide treatments, Mn treatments, and cultivars.

Clearly, large amounts of fertilizer N at planting decreased protein concentration and increased oil concentration. Together these consistent increases and decreases affected the overall protein/oil ratio. Fig. 3 shows a one-to-one graph of the protein/oil ratio of the PlusN treatment plotted against the ZeroN treatment. For the irrigated environment all 17 data points fell on or below the 1:1 line. For the non-irrigated environment, four data points fell above the 1:1 line. There was no commonality among the four data points except that they were all from the 2002 experiment. When considered over all management practices, fertilizer N significantly ($P < 0.0001$) reduced the protein/oil ratio in the irrigated (4.7% reduction, 1.85 ZeroN versus 1.76 PlusN) and the non-irrigated (4.6% reduction, 1.84 ZeroN versus 1.75 PlusN) environments. There was no significant ($P = 0.9111$) difference in the response to fertilizer N between irrigation environments (Table 2).

Detailed analysis of seed yield from this study is described by Ray et al. (2006). However, a brief synopsis of the N treatments is presented here and supported by the data summarized in Table 3. Analysis over all management practices showed that seed yield was significantly increased in response to N in both irrigated ($P < 0.0001$) and non-irrigated ($P < 0.0001$) environments. Fertilizer N application increased seed yield from 4184 to 4507 kg ha^{-1} (7.71%) in the irrigated and from 2817 to 3255 kg ha^{-1} (15.53%) in the non-irrigated environment. There was a significant difference ($P = 0.0463$) in the response to

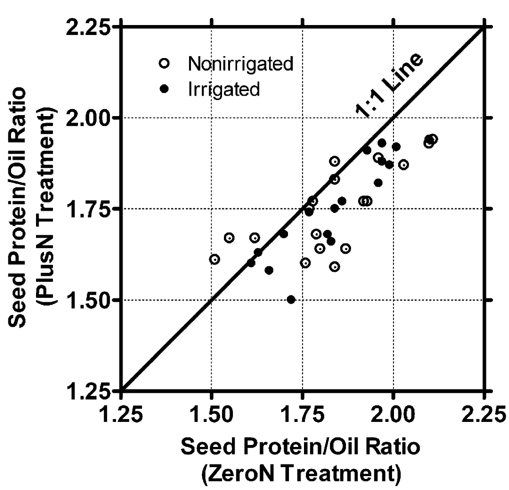

Fig. 3. One-to-one graph of protein/oil ratio in the ZeroN treatment (*x*-axis) plotted against protein/oil ratio in the PlusN treatment (*y*-axis) across all management practices (years, cultivars, herbicide, and Mn treatments) and irrigation environments (irrigated and non-irrigated). Each data point represents paired samples (i.e. the value of both a ZeroN and PlusN sample).

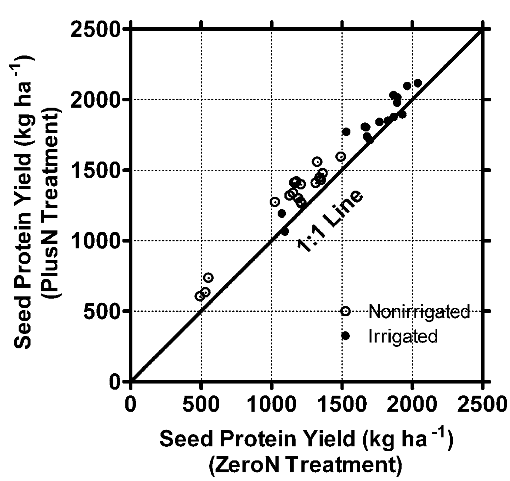

Fig. 4. One-to-one graph of protein yield (kg ha^{-1}) in the ZeroN treatment (*x*-axis) plotted against protein yield in the PlusN treatment (*y*-axis) across all management practices (years, cultivars, herbicide, and Mn treatments) and irrigation environments (irrigated and non-irrigated). Each data point represents paired samples (i.e. the value of both a ZeroN and PlusN sample).

Table 3
Seed yield (kg ha^{-1}) over all cultivars, herbicide and Mn treatments summarized by irrigation environment and fertilizer N treatment and fertilizer N treatment within irrigation environment

	Seed yield (average) kg ha^{-1}	Standard error	N	Minimum (kg ha^{-1})	Maximum (kg ha^{-1})
Irrigated	4346	58	136	2636	5514
Nonirrigated	3036	62	144	1206	4144
PlusN[a]	3863	80	140	1310	5514
ZeroN[b]	3481	81	140	1206	5064
Irrigated					
PlusN	4507	80	68	2778	5514
ZeroN	4184	81	68	2636	5064
Nonirrigated					
PlusN	3255	88	72	1310	4144
ZeroN	2817	81	72	1206	3582

[a] PlusN treatment = 290 kg ha^{-1} in 2002, 310 kg ha^{-1} in 2003 and 360 kg ha^{-1} in 2004.
[b] ZeroN treatment (no fertilizer N).

fertilizer N between the irrigated and non-irrigated environment.

Relationships among seed yield, protein concentration, and oil concentration were examined using correlation and regression analysis. Over all management practices, irrigation environments and N treatments, there was a significant ($P = 0.0003$) negative correlation ($r = -0.42$) between protein and oil concentration. There was no significant difference in the response between N treatments (i.e. the regression slopes were not significantly different, $P = 0.5623$, pooled slope = -0.26). There was no significant correlation between oil concentration and seed yield in either the PlusN treatment ($P = 0.8541$) or ZeroN treatment ($P = 0.9988$). However, for protein concentration, over all management practices, irrigation environments and N treatments, there was a significant positive correlation between protein and yield ($r = 0.45$, $P = 0.0001$) and the response was the same in both N treatments (no significant difference in slope, $P = 0.9896$, pooled slope = 216.5).

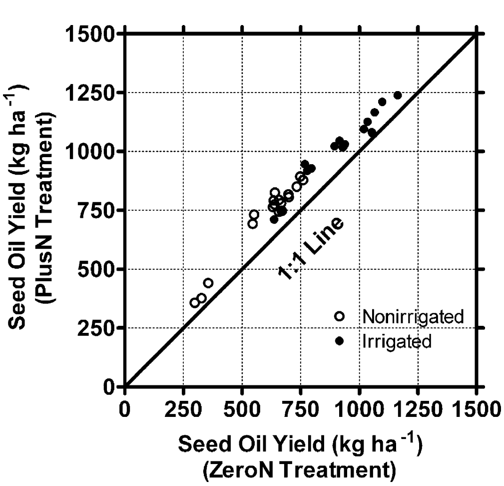

Fig. 5. One-to-one graph of oil yield (kg ha^{-1}) in the ZeroN treatment (*x*-axis) plotted against oil yield in the PlusN treatment (*y*-axis) across all management practices (years, cultivars, herbicide, and Mn treatments) and irrigation environments (irrigated and non-irrigated). Each data point represents paired samples (i.e. the value of both a ZeroN and PlusN sample).

In the irrigated environment, protein yield ranged from 1061 to 2114 kg ha^{-1} and in the non-irrigated environment from 496 to 1591 kg ha^{-1}. Fig. 4 shows a one-to-one graph of the protein yield of the PlusN treatment plotted against the ZeroN treatment over all years, management practices, N treatments and irrigation environments. In the irrigated environment, all but two data points fell above the 1:1 line and in the non-irrigated environment, all data points fell above the line. When considered over all management practices (years, cultivars, herbicides and Mn treatments) there was a significant ($P < 0.0001$) 5.0% increase in protein yield in the irrigated environment (1708 g kg^{-1} ZeroN versus 1795 g kg^{-1} PlusN) with the addition of fertilizer N. In the non-irrigated environment, there was a much larger significant ($P < 0.0001$) 12.7% increase (1126 g kg^{-1} ZeroN versus 1269 g kg^{-1} PlusN) with fertilizer N. There was a significant difference ($P = 0.0322$) in the protein yield response to fertilizer N between irrigation environments (Table 2).

Oil yield also increased with fertilizer N applied at planting (Fig. 5). In the irrigated environment, oil yield ranged from 639 to 1236 kg ha^{-1} and in the non-irrigated environment from 300 to 891 kg ha^{-1}. For oil yield, all data points in both the irrigated and non-irrigated environment fell above the 1:1 line (Fig. 5). In the irrigated environment, when analyzed over all management practices, there was a significant ($P < 0.0001$) increase of 9.9% (928 versus 1020 kg ha^{-1}) in oil yield with the application of fertilizer N. In the non-irrigated environment, the increase in oil yield was also significantly ($P < 0.0001$) greater (18.9%, 609 versus 724 kg ha^{-1}, ZeroN versus PlusN) in response to fertilizer N. However, there was no significant ($P = 0.0819$) difference in the response between irrigation environments (Table 3).

4. Discussion

The substantially lower yield in the non-irrigated environment compared to the irrigated environment (average 3036 versus 4346 kg ha^{-1}, Table 3) indicates that water deficits were severe enough to adversely affect yield. Similarly, the greater yield in the PlusN treatment compared to the ZeroN treatment (average 3863 versus 3481 kg ha^{-1}, Table 3) indicates that fertilizer N had a major impact on yield. The range of yields obtained over the 3 years of this study represent a wide range of management practices (including year-to-year weather fluctuations and cultivar differences) irrigation environments and N applications. The wide diversity of conditions under which the effect of fertilizer N was evaluated allows for broad analysis and interpretation. Our primary goal in this report was to examine the effect of large amounts of fertilizer N applied at planting on protein and oil concentration in soybean seed.

With nodulated soybean, Weber (1966) showed a slight increase in protein concentration and a slight decrease in oil concentration with fertilizer N, whereas, Purcell et al. (2004) showed the opposite. Both of these studies evaluated one nodulating genotype each. In this study, we evaluated eight different cultivars (Table 1) in irrigated and non-irrigated environments with large amounts of fertilizer N applied at planting. The results were in agreement with Purcell et al. (2004) and showed that fertilizer N significantly reduced protein concentration (by 1.9% non-irrigated and 2.7% irrigated) and significantly increased oil concentration (by 2.2% non-irrigated and 2.7% irrigated) in soybean seed (Figs. 1 and 2). This resulted in a significant negative ($r = -0.42$) correlation between protein and oil concentrations considered over both irrigated and non-irrigated environments and with or without large amounts of fertilizer N applied at planting. A negative correlation between protein and oil is in agreement with previous reports (selected references: Johnson et al., 1955; Hartwig and Hinson, 1972; Chung et al., 2003). Overall, the protein/oil ratio significantly decreased (by 4.7% irrigated and 4.6% non-irrigated) with the application of large amounts of fertilizer N at planting (Fig. 3). These changes reflect the overall decrease in protein and the simultaneous increase in oil concentration with fertilizer N. The mechanism by which increased soil N differentially affected seed protein and oil concentrations is not clear. However, it is likely a combination of reduced assimilation costs (soil N versus atmospheric N_2) and the differential bioenergetics of protein and oil synthesis. Nitrate is known to be the primary signal for regulating nitrate assimilation (see Coruzzi and Bush, 2001; Crawford, 1995), however, its possible role in regulating seed protein and oil concentrations are unknown.

Oil yield was significantly increased (by 9.9% irrigated and 18.9% non-irrigated) with large amounts of N applied at planting (Fig. 5). Although protein concentration (g kg^{-1}) decreased with large amounts of fertilizer N (relative to no fertilizer N; Fig. 1) applied at planting, the overall protein yield (g ha^{-1}) increased (Fig. 4) by 5.0% in the irrigated environment and by 12.7% in the non-irrigated environment because of the large increase in seed yield as a result of the fertilizer N. The large increases in seed yield compensated for the decline in protein concentration. In many cases, it has also been reported that as protein concentration increases, yield decreases (Burton, 1987; Wilcox and Cavins, 1995; Chung et al., 2003). However, our results indicated an overall significant positive correlation ($r = 0.45$, $P = 0.0001$) between yield and protein concentration and there was no significant difference between the ZeroN and PlusN treatments. The previously cited negative correlations between protein and yield were generally found within genetic populations whereas we evaluated the response across management practices (years, cultivars, herbicide treatments, and Mn treatments), irrigation environments and fertilizer N. Nonetheless, understanding the mechanisms controlling the relationship between environment and seed protein concentration may facilitate the breaking of the commonly cited negative relationship between increased protein and yield.

Irrigation environment (irrigated versus non-irrigated) showed no significant difference in the effect large amounts of N applied at planting had on protein concentration, oil concentration, the protein/oil ratio, or oil yield. However, there was a significant difference in effect on protein yield. While protein yield was increased in both environments, the increase was much greater in the non-irrigated environment. Within both irrigation environments, protein concentration (g kg^{-1}) decreased and oil concentration (g kg^{-1}) increased in the added fertilizer N treatment compared to the zero added N treatment. This resulted in a decreased protein/oil ratio. However, both protein yield and oil yield (kg ha^{-1}) increased with the application of fertilizer N at planting due to the substantially increased seed yield (kg ha^{-1}). A small but significant positive correlation was observed between protein concentration and seed yield in both irrigation environments. How fertilizer N affected the balance of protein and oil was not determined in this study. Physiological and molecular comparison of the pathways of ureide and nitrate metabolism and the signaling mechanisms regulating both processes may lead to a greater understanding of the factors governing protein and oil concentration in soybean.

Acknowledgments

The authors appreciate the technical assistance provided by Sandra Mosley and Gus Eiffling as well as the resources provided by the Delta Research and Extension Center. Special appreciation is acknowledged to Debbie Boykin for statistical advice. This research was partially supported by United Soybean Board Project #4244.

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